OFF CGA

Sheet 1 of 1
Appl. No. 10/719,978; Filed: Nov 24, 2003
Dkt No. 1530.0550001/JUK/JCI; Group Unit: 1645
Inventors: Budahazi, et al Tel: 202-371-2600
Title: Process for Purification of Plasmid DNA
Replacement Sheet

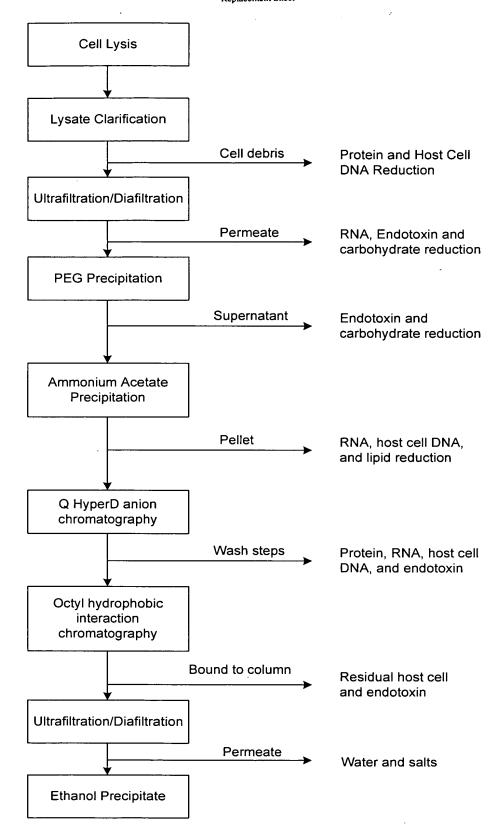


FIG. 1

Sheet 1 of 1
Appl. No. 10/719,978; Filed: Nov 24, 2003
Dkt No. 1530.0550001/IUK/JCI; Group Unit: 1645
Inventors: Budahazi, et al Tel: 202-371-2600
Title: Process for Purification of Plasmid DNA
Originally submitted Figure 1

Figure 1. Process Flow Chart

The cells are resuspended in buffer using a static mixer and a closed vessel. Cell lysis is completed by a modification of the alkaline lysis method followed by neutralization. Gentle mixing is completed using a static mixer in continuous re-circulation mode.

Removal of cell debris and clarification is accomplished by diatomite aided depth filtration.

After clarification, the lysale is concentrated 10-15 fold using hollow fiber ultrafiltration followed by a buffer exchange.

The concentrated nucleic acid is selectively precipitated using PEG for further enrichment and concentration.

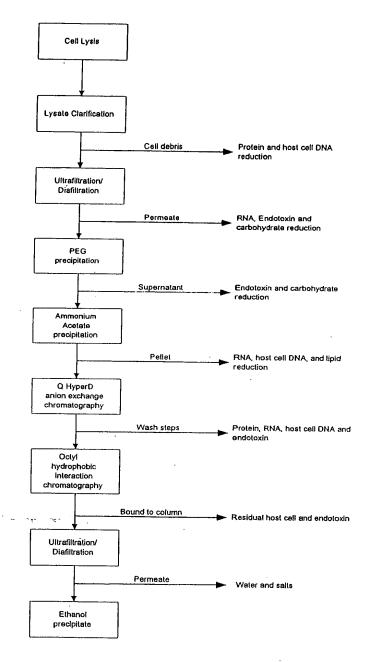
The PEG pellet is collected by centrifugation and dissolved in buffer. Ammonium acetate is added to the solution to selectively precipitate contaminants which are removed by centrifugation. The supernatant is IPA precipitated and stored at -20 until chromatography step. STOP POINT

The IPA precipitated nucleic acid is pelleted by centrifugation. The pellet is dissolved in column buffer A. 0.22um filtered and loaded onto the column. After loading there are intermediate step washes before product elution.

The HyperD elution peak is diluted 1:1 with 3M ammonium sulfate, 0.22um filtered then loaded onto the HIC column. Under the load conditions product flows through in the vold volumne; residual contaminants bind to the column.

The purified pDNA is concentrated followed by a buffer exchange to remove the ammonium acetate.

The purified DNA is ethanol precipitated and stored at -20 C until to bulking.



Sheet 1 of 1
Appl. No. 10/719,978; Filed: Nov 24, 2003
Dkt No. 1530.0550001/JUK/JCI; Group Unit: 1645
Inventors: Budahazi, et al Tel: 202-371-2600
Title: Process for Purification of Plasmid DNA
Annotated Marked-Up Drawing

Figure 1. Process Flow Chart

The cells are resuspended in buffer using a static mixer and a closed vessel. Cell tysis is completed by a modification of the alkaline tysis method followed by neutralization.— Gentle mixing is completed using a static mixer in continuous re-circulation mode.

- Removal of cell-debris and clarification is accomplished by distants aided depth filtration.
- After clarification, the tysale is concentrated 10-15-fold using hollow fiber ultrafiltration followed by a buffer -- exchange.
- The concentrated nucleic sold is selectively-precipitatedusing PEG for further enrichment and concentration.
- The PEC pellet is collected by centrifugation and dissolved in buffer. Ammonium acotate is added to the soldion to selectively precipitate contaminants which are removed by contrifugation. The supernatant is IPA precipitate and stored at 20 until chromategraphy step.
- -The IPA precipitated nucleic acid is pelleted by -centrifugation. The pellet is dissolved in column buffer A,
 -0.22um filtered and loaded onto the column. After loading—
 there are informediate step washes before product—
 elddian—
- The HyperD elution peak is diluted 1:1 with 3M-ammonium sulfate, 0.22um filtered then leaded ento the
 Hife column. Under the lead conditions product flows—
 Hirough in the volid volumne; recidual contaminants bind te—
- The purified pDNA is concentrated followed by a buffer exchange to remove the ammonium acetate.
- -The purified DNA is ethanol precipitated and sterod at 20-0 until to bulking.-

